REMARKS/ARGUMENTS

The final Office Action of October 28, 2008, has been carefully reviewed and these remarks are responsive thereto. Claims 1-3, 5, 6, 9-11, 13-15, 17-19, 21-25, and 27-29 were pending. Claims 1-3, 5, 6, 9-11, 13-15, 17-19, 21, 27 and 29 were rejected, and claims 22-25 and 28 were objected to. By this response, claims 21, 22, 24 and 28 have been amended, and claims 9, 10, 13, 14, and 18 have been canceled. Claims 4, 7-8, 12, 16, 20, and 26 were canceled in a prior response. Thus, claims 1-3, 5, 6, 9-11, 13-15, 17-19, 21-25, 27-29 remain pending. No new matter has been added to the application.

Priority

The Office Action acknowledged applicant's claim for foreign priority under 35 U.S.C. 119 (A)-(D) to the foreign application EPO 02014991.0, but deemed that the disclosure of the foreign application EPO 02014991.0 fails to provide adequate support or enablement for one or more claims of this application. The Office Action stated that the instant claims disclose a delivery system comprising the NCAM Ig loop domains I, II, and III, wherein the delivery system further comprises an integrase, wherein the integrase is from the phiC31 bacteriophage. The Office Action stated that the application EPO 02014991.0 does not provide support for the use of the NCAM Ig loop domains I, II, and III or for the use of any integrase. The Office Action stated, therefore, the priority date for these embodiments is considered to be the filing date of the PCT/CH03/00453, i.e., 07/08/2003.

Applicant disagrees with the priority determination expressed in the Office Action. EPO 02014991.0 does indeed provide support for the use of the NCAM Ig loop domains I, II, and III, as well as the use of an integrase. Specifically, see page 2, lines 5 through page 4, line 13 of EPO 02014991.0. Thus, the priority date of the present application is July 10, 2002, the date of filing of EPO 02014991.0.

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Information Disclosure Statement

The Information Disclosure Statement filed 1/10/2005 included a reference, DE 100 56 136 with no English translation. Applicant states that an English translation of DE 100 56 136 can be found at U.S. Patent Publication No. 2004/0191303.

Claim Rejections Under 35 U.S.C. §175(c)

Claims 22-25 and 28 were objected to under 36 CFR 1.75(c) as being improper form because a multiple dependent claim cannot serve as a basis for any other multiple dependent claims, either directly or indirectly. By this response, claims 22, 24, and 28 have been amended so that they no longer depend from another multiple dependent claim, either directly or indirectly. Claim 25 depends from claim 24. The Applicant respectfully requests that claims 22-25 and 28 be considered on the merits.

Claim Rejections Under 35 U.S.C. §112

Claims 1-3, 5, 6, 9, 10, 13, 14, 17, 18, 21, and 27 were objected to under 35 U.S.C. 112, first paragraph as failing to comply with the written description requirement. Specifically, the term "a DNA integrase activity" was considered to be new matter.

Applicant respectfully disagrees with this rejection. The DNA integrase activity disclosed in the application is DNA integrase activity encoding a protein, not necessarily being a protein. *See e.g.*, EPO 02014991.0 at page 3, lines 6-15, and page 4, lines 17-18, page 5, lines 30-33. Thus, the rejection should be withdrawn.

Claim Rejections Under 35 U.S.C. §103(a)

Claims 1-3, 5, 6, and 27 were rejected under 35 U.S.C. 103(a), as being unpatentable over Poulsen et al. (PGPUB 2005/0037445), in view of each Maurer et al. (Expert Opinion Biol Ther, 2001, 1:923-947), Groth et al. (Proc. Natl. Acad. Sci. USA, 2000, 97:5995-6000), Schreier et al. (J. Biological Chemistry, 1994, 269:9090-9098), and Ranheim et al. (Proc. Natl. Acad. Sci. USA, 1996: 93:4071-4075).

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Claims 1-3, 5, 6, 9, 10, 13, 14, 17, 18, 21, and 27 were rejected under 35 U.S.C. 103(a), as being unpatentable over Poulsen et al., taken with each Maurer et al., Groth et al., Schreier et al., and Ranheim et al., in further view of each Sato et al. (J. Drug Target., 2001, 9:201-207) and Gosselin et al. (Bioconjugate Chem., 2001, 12:989-994).

Claims 1, 2, 5, 6, and 27 were rejected under 35 U.S.C. 103(a), as being unpatentable over Murphy (U.S. Patent No. 6,635,476) in view of each Poulsen et al., Ranheim et al., and Groth et al.

Claim 29 was rejected under 35 U.S.C. 103(a), as being unpatentable over Poulsen et al., in view of each Maurer et al., Smith et al. (U.S. Patent No. 6,329,501), and Charlton et al. (Developmental Biology, 2000, 221: 112-119).

Rejections based on Poulsen as the primary reference

Poulsen discloses targeting complexes that are capable of being internalized into cells. The targeting complexes include at least one binding partner to associate with a cell surface molecule, and a bioreactive species. (See paragraphs [0079] and [0087] of Poulsen) As recognized by the Examiner, Poulsen does not specifically teach liposomes comprising DNA as a bridge between a nucleic acid and a targeting moiety. In other words, Poulsen does not teach liposomes comprising DNA in their internal compartment and having the cell adhesion molecule NCAM or a fragment thereof. Further, Poulsen is silent regarding targeting complexes that comprise "a DNA integrase activity or a molecule encoding such a DNA integrase activity" as claimed in claim 1.

None of Maurer, Groth, Schreier nor Ranheim remedy the deficiencies of Poulsen with respect to liposomes comprising DNA integrase activity. Maurer is directed to a review of liposomes for drug delivery and discusses only conventional drugs, DNA and pDNA (See Abstract and page 936, column 1 through page 941, column 1 of Maurer). Maurer is thus completely silent regarding DNA integrase activity. Groth is directed to integrase from phi31 to carry out site-specific integration in human cells, but is totally unrelated to NCAM. Ranheim is directed to the interaction of neural cell adhesion molecules (NCAM) on two different cells and is totally unrelated to DNA integrase activity (See Abstract of Ranheim). Schreier is directed to

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glycosylphosphatidylinositol-anchored proteins for use as targeting molecules for liposomes, and is entirely silent regarding DNA integrase activity (See Abstract of Schreier).

Consequently, it would not have been obvious for one of skill in the art to develop the invention of claim 1 merely from the disclosures of Poulsen, Maurer, Groth, Schreier and Ranheim. Claim 1 is therefore patentable over Poulsen in view of Maurer, Groth, Schreier and Ranheim. The Office Action is silent regarding how the application of the *Graham* factors would demonstrate the obviousness of claim 1.

One ordinary skill in the art at the time the invention was made would not have been motivated to make the proposed modifications of Poulsen because it was ***only in the Applicant's disclosure*** that it was recognized that liposomes which comprise in their internal compartment a pharmaceutical agent and which have linked to their external surface the cell adhesion molecule NCAM or a fragment thereof, wherein said pharmaceutical agent is DNA and said delivery system comprises a DNA integrase activity or a molecule encoding such a DNA integrase activity as claimed in claim 1 could be useful.

One ordinary skill in the art at the time the invention was made would not have been motivated to make the proposed modifications of Poulsen because it was ***only in the Applicant's disclosure*** that it was recognized that liposomes which comprise in their internal compartment a pharmaceutical agent and which have linked to their external surface the cell adhesion molecule NCAM or a fragment thereof, wherein said delivery system comprises DNA that encodes the human dystrophin protein as claimed in claim 29 could be useful.

The present case is similar to *Sanofi-Synthelabo v. Apotex Inc.*, 550 F3d 1075 (Fed. Cir. Dec. 12, 2008). In *Sanofi*, the Federal Circuit affirmed a lower court holding of nonobviousness. In doing so, the Federal Circuit stated that "[t]he determination of obviousness is made with respect to the subject matter as a whole, not separate pieces of the claim," citing *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1734 (2007). The Federal Circuit further held that "[f]or chemical compounds, the structure of the compound and its properties are inseparable considerations in the obviousness determination. *See In re Sullivan*, 498 F.3d 1345, 1353 (Fed. Cir. 2007); *In re Papesch*, 315 F.2d 381, 391 (CCPA 1963)." In *Sanofi*, while it was known that different

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enantiomers of a compound "can exhibit different biological activities," it was also clear from the record that it was not predictable whether such differences, if any, would be weak, moderate, or strong, or how they would be manifested." The record demonstrated that there was no known scientific principle that allowed prediction of the degree to which stereoisomers would exhibit different levels of therapeutic activity and toxicity. The record also demonstrated that two properties (i.e., activity and toxicity) were more likely to be positively correlated, such that a reduction in toxicity would be expected also to reduce the beneficial activity. Witnesses also explained that it was known that for compounds whose biological activity is delivered through metabolism within the body, the acid environment in the stomach or other metabolic processes often restores the racemic state, thereby removing any potential benefit of a separated enantiomer. On the basis of the trial evidence, the district court found that a person of ordinary skill in this field would not reasonably have predicted that the dextrorotatory enantiomer would provide all of the antiplatelet activity and none of the adverse neurotoxicity. The Federal Circuit held that clear error had not been shown in this finding, and in the conclusion of nonobviousness based thereon, again citing *Papesch*, 315 F.2d at 391 (a chemical compound and its properties are inseparable).

In the present case, it is clear from the record that it was not predictable whether modifications of Poulsen as proposed in the Office Action would exhibit different biological activities, and it was also not predictable whether any differences, if any, would be weak, moderate, or strong, or how they would be manifested. The record demonstrates that there was no known scientific principle that allowed prediction of the degree to which different forms of liposomes would exhibit different levels of therapeutic activity. On the basis of this evidence, a person of ordinary skill in this field would not reasonably have predicted that the claimed invention would provide the results of the present invention. The results of the present invention are shown in the accompanying Scientific Report, entitled "Development of Novel Non-Viral Vectors for Gene Therapy of Muscular Diseases," of which the Applicant is an author.

The accompanying Scientific Report shows that the use of NACM efficiently enhances the uptake of a plasmid into muscle cells. In this specific experiment, the P2 peptide, being

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derived from the second Ig domain of NCAM, was coupled to biotin. Furthermore, biotin was also linked to the outer surface of the liposome. By using streptavidin, which binds to biotin, P2 was linked to the liposome. When exposed to the cell line (C2C12, ATCC Number CRL-1772), the P2 peptide was able to mediate the uptake of plasmid DNA, coding for Renilla Luciferase, from liposomes. As a result, the transformed cells expressed Renilla Luciferase and gave a positive signal in a subsequent luciferase assay. However, a liposome without a targeting molecule was not able to efficiently transform the cell line; nor did the sample where the P2:Streptavidin ratio reached 2.5:1. In the latter case, P2 saturates the binding sites of streptavidin, hence the coupling of P2 to the liposome via streptavidin-biotin-interaction is not possible or occurs only in an ineffective manner.

Sato and Gosselin do not remedy the deficiencies in the other cited references as to claims 1 and 29. As in *Sanofi* and *Papesch*, the present invention is nonobvious over the prior art. The pending dependent claims depend from claim 1 and are patentable for at least the same reasons as claim 1 is patentable and for the additional features recited therein.

Applicants respectfully request that if the §103 rejection is maintained, or if further rejections are set forth, that the Office provide an analysis of obviousness that considers the Graham factors so the Applicants may more readily respond to any assertions of obviousness.

Rejection based on Murphy as the primary reference

Claims 1, 2, 5, 6, and 27 were rejected under 35 U.S.C. 103(a), as being unpatentable over Murphy (U.S. Patent No. 6,635,476) in view of each Poulsen et al., Ranheim et al., and Groth et al. As discussed above, claim 1 includes the features that "said pharmaceutical agent is DNA and said delivery system comprises a DNA integrase activity or a molecule encoding such a DNA integrase activity." Murphy is directed to targeted vectors "that are complexed to a targeting moiety by coordinate covalent linkages mediated by a transition metal ion" (Abstract of Murphy). Poulsen and Ranheim are discussed above as lacking disclosure related to liposomes comprising DNA integrase activity, and Murphy is also silent regarding DNA integrase activity.

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Groth is directed to integrase from phi31 to carry out site-specific integration in human cells, but is totally unrelated to NCAM.

In the present case, it is clear from the record that it was not predictable whether modifications of Murphy as proposed in the Office Action would exhibit different biological activities, and it was also not predictable whether any differences, if any, would be weak, moderate, or strong, or how they would be manifested. The record demonstrates that there was no known scientific principle that allowed prediction of the degree to which different forms of liposomes would exhibit different levels of therapeutic activity. On the basis of this evidence, a person of ordinary skill in this field would not reasonably have predicted that the claimed invention would provide the results of the present invention. The results of the present invention are shown in the accompanying Scientific Report, entitled "Development of Novel Non-Viral Vectors for Gene Therapy of Muscular Diseases," of which the Applicant is an author. As in *Sanofi* and *Papesch*, the present invention is nonobvious over the prior art.

Accordingly, claim 1 is patentable over Murphy in view of Poulsen and Ranheim. The dependent claims depend from claim 1 and are patentable for at least the same reasons as claim 1 and for the additional features recited therein. The Applicant respectfully requests withdrawal of the 35 U.S.C. § 103(a) rejections.

Applicants respectfully request that if the §103 rejection is maintained, or if further rejections are set forth, that the Office provide an analysis of obviousness that considers the Graham factors so the Applicants may more readily respond to any assertions of obviousness.

CONCLUSION

All rejections having been addressed, the Applicant respectfully submits that the instant application is in condition for allowance, and respectfully requests prompt notification of the same. If there are any questions, the examiner is invited to contact Applicant's undersigned representative at the number noted below.

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Respectfully submitted,

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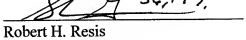
Dated: April 28, 2009

By: 

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SCIENTIFIC REPORT

DEVELOPMENT OF NOVEL NON-VIRAL VECTORS FOR A GENE THERAPY OF MUSCULAR DISEASES

09.08.2005

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Keywords:

gene therapy, liposomes, myopathies, apoptosis, bcl, integrase, NCAM

Stability of the lipovectors

Gene therapy trials with non-viral vectors are often hampered by low efficiency and high toxicity of the vectors. The new generation of cationic lipovectors displays improved gene transfer capacity and reduced toxicity, but the efficiency is still limited due to rapid clearance from the blood stream. The ideal lipovector shows a long circulation time in the blood stream, has a low affinity for the mononuclear phagocytic system, navigates to the target tissue, is highly efficient in delivering nucleic acids to cells, and does not lead to cell death [1].

We showed previously that our home-made lipovectors, based on DOPE and DDAB lipids, were efficient in transfection of rodent and human muscle cells. To analyse the stability of our lipovectors, we loaded them with the reporter plasmid pSCT1/lac-Z. The enzyme β-galactosidase is transcribed from this plasmid under the control of the CMV promotor. The expression of the enzyme can be easily quantified with a fluorescence-based enzymatic assay. Increasing amounts of plasmid-loaded lipovectors were incubated in freshly isolated human blood. More than 20% of the lipovectors could be retrieved from the plasma fraction in an active form (figure 1). Within a concentration range from 0.45 µg DNA per ml of blood to 0.90 µg of DNA per ml of blood, we recovered the maximal amount of active lipovector-associated plasmid DNA.

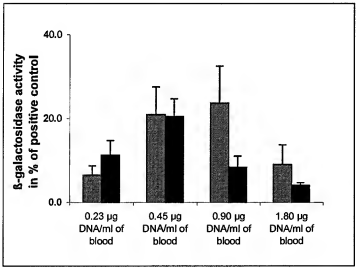

Figure 1. Stability of our home-made lipovectors in human blood. Lipovectors loaded with increasing amounts of plasmid DNA were either directly applied to the cultured cells (positive control), or they were incubated at RT for 30 minutes with 10 ml of human blood, prior to application to the cultured cells. The percentage of lipovector activity recovered from the plasma fraction is shown. Gray and black columns display results obtained with two individual blood donors.

Toxicity of the lipovectors

Transfection initiates the cellular suicide program in a considerable number of cells. On the one hand this is due to the toxicity of the lipids of the lipovectors, and on the other hand this is due to the accumulation of transgenic gene products. Retardation of apoptosis may thus

significantly enhance transfection efficiency, simply by transiently seizing the suicide program of the transfected cells.

In order to test this hypothesis, we incubated muscle cells with tunicamycin, a well described trigger of apoptosis. Prior to the tunicamycin treatment, we transfected the cells with two recently described anti-apoptotic factors, bcl_2 and bcl_{xl}, together with a reporter plasmid. Expression of the reporter plasmid was only observed when either of the two anti-apoptotic factors, or both, were co-transfected with the reporter plasmid. The most efficient prevention of apoptosis was achieved with co-transfection of bcl_{xl}. The massive increase in survival rate upon expression of bcl_{xl} indicates that simultaneous introduction of this factor decelerates cell death (figure 2). In a next step, we will co-transfect cells with mRNA encoding the anti-apoptotic factors. This should lead to a transient halt of the apoptotic program, thus avoiding oncogenic effects due to permanent elimination of apoptosis. In previous analyses, we could show that cells transfected with a reporter mRNA indeed expressed the transgenic reporter only for a short period of time, whereas the same reporter gene encoded as DNA on a plasmid was active for substantially longer time periods.

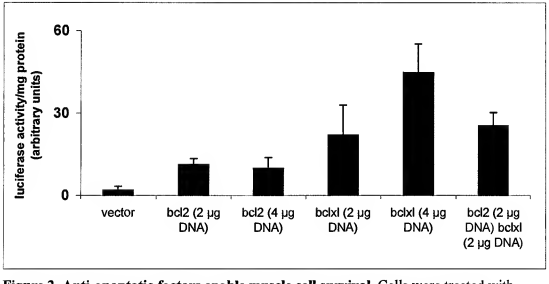

Figure 2. Anti-apoptotic factors enable muscle cell survival. Cells were treated with the apoptosis-inducing agent tunicamycin. All cell samples have been transfected with identical amounts of reporter plasmid (2.5 µg) concomitantly with the indicated amounts of plasmids encoding the anti-apoptotic factors. We detected significant luciferase activity exclusively in cells that expressed the anti-apoptotic factors.

Integration into the host genome

The selective integration of foreign DNA into the genome of muscle cells is fundamental for long-term correction of disease status. We are addressing this issue by using the

bacteriophage-derived integrase C31. This integrase uses attachment sites for genomic integration. The attachment sites are comprised of sequence stretches of around 35 base pairs. There exist only limited numbers of these well-defined sequence stretches throughout the whole mammalian genome, rendering integration via this enzyme site-specific and therefore safer than randomn retroviral integration (reviewed by [2]).

For a first analysis of efficiency of integration, we cloned the C31 integrase downstream of a CMV promoter and attached in frame to the 3' end of the coding region the nuclear localization signal (NLS) of the SV40 virus large T-antigen. To monitor expression levels, we also fused the 3' end of the coding region to a heme agglutinin (HA-) tag. These constructs were tested for integrating efficiency by using two different reporter plasmids. The first plasmid is composed of the attachment site that is recognized by the integrase, fused to the coding region for a reporter gene (luciferase). The second plasmid carries the promoter for the luciferase gene. In addition, this second plasmid contains the sequence stretch used by the integrase for recombination with the attachment site of the luciferase-encoding plasmid. Active C31 integrase recombines these two plasmids and positions the CMV promoter to the 5' end of the luciferase gene, thus enabling transcription. The amount of expressed luciferase is proportional to the activity of the C31 integrase. Using these constructs, we were able to show that our diverse C31 integrase constructs increased luciferase activity more than 50 fold. We will now analyse the efficiency of our C31 integrase constructs in mediating integration into the mammalian genome. For this purpose, we will co-transfect into muscle cells the C31 integrase and a reporter plasmid that encodes the resistence gene for neomycin. Successful genomic integration will be monitored by cell survival in neomycin-enriched medium and will be compared to the survival of cells that were transfected with the reporter plasmid only. We expect maximal cell survival rates upon co-transfection of the reporter plasmid with the integrase that harbours the NLS at its 3' end.

In vivo muscle targeting

Specific targeting may increase accumulation of lipovectors in the desired organ by a factor of 3-4 fold. Such an increase in local lipovector concentration elevates the probability of successful transfection. We investigated muscle cell targeting with the neural cell adhesion molecule (NCAM-) - derived peptide P2, which mimics NCAM-homophilic binding [3] . As NCAMs are expressed in regenerating muscles [4], targeting NCAM-expressing cells with lipid particles decorated with the peptide P2 appeares to be a suitable strategy towards favoring an accumulation of lipovectors in muscular tissue.

To this end, in a cooperation with Dr. Schwamberger from the University of Salzburg in Austria, we developed lipid vesicles that were functionalised with the peptide P2. The lipid vesicles contain reporter plasmids, the expression of which can be easily monitored in single cells. Binding to NCAM-expressing muscle cells and subsequent internalization of the vesicles will be analysed in NCAM-expressing myotubes as well as in non-muscle cells that were transfected with a NCAM-encoding expression plasmid. We monitor NCAM expression with the monoclonal antibody 5B8, which recognizes the mouse form of NCAM. Furthermore, we established a suitable protocol for detection of P2 binding to NCAM-expressing cells. For this purpose, the P2 peptides were covalently coupled to biotin. These biotinylated peptides have been visualized with fluorescently labelled Streptavidin. Differentiated muscle cells bind the biotinylated peptides (figure 3), suggesting that they can be used for targeting purposes. Currently we are working on the optimization of the specificity of the peptide.

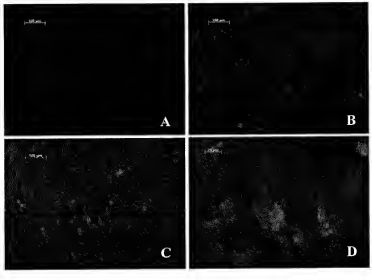

Figure 3. Biotinylated P2 peptide binds to differentiated muscle cells. Mouse muscle cells were differentiated in medium containing 2% horse serum, fixed and incubated for 1h with varying amounts of biotinylated NCAM-derived peptide P2. The biotinylated peptide was then visualized with TRITC-labeled streptavidin. A) no peptide. B) 5 µg/ml of P2 peptide. C) 50 µg/ml of P2 peptide. D) 50 µg/ml of P2 peptide – higher magnification.

Summary

- We created a stable and efficient lipovector
- A substantial fraction of our lipovector retains its activity upon incubation with considerable amounts of human blood

- We were able to abrogate apoptosis in muscle cells with specific anti-apoptotic factors. These factors were encoded on plasmids that were delivered to the muscle cells with our lipovectors
- We showed that the C31 integrase is very efficient in mediating site-specific integration, thus enabling a permanent repair of dys-functional genes
- The NCAM-derived peptide P2 turned out to be a promising tool for muscle cell targeting

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